

Figure 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide →
~~~~~ ~~~~~~  
1    **AAGCTTGC**CG CC    ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V<sub>L</sub> →  
61    V I I A R G Q I V L S Q S P A I L S A S  
GTCATAATTG CCAGAGGACA ATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

121    P G E K V T M T C R A S S S V S Y M H W  
CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
~~~~~  
181 Y Q Q K P G S S P K P W I Y A P S N L A
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATGCCCATC CAACCTGGCT

241 S G V P A R F S G S G S G T S Y S L T I
TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

301 S R V E A E D A A T Y Y C Q Q W S F N P
AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTJTAACCCA

(Gly₄Ser)₃ Linker
P T F G A G T K L E L K G G G S G G G
CCCACGT'CG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT

2H7 V_H →
421 G S G G G G S S Q A Y L Q Q S G A E L V
GGATCTGGAG GAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC T'GAGCTGGTC

481 R P G A S V K M S C K A S G Y T F T S Y
AGGCCTGGGG CCTCAGTGAA CATGTCCCTGC AAGGCTTCTG GCTACACATT TACCAAGTTAC

541 N M H W V K Q T P R Q G L E W I G A I Y
AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT

601 P G N G D T S Y N Q K F K G K A T L T V
CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

661 D K S S S T A Y M Q L S S L T S E D S A
GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGC

721 V Y F C A R V V Y Y S N S Y W Y F D V W
GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGTCTGG

Figure 1 B

BcI I
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCT**GAT** CAGGAGCCCA AATCTGTGA CAAAACTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCAC CGTGCCAGC ACCTGAACTC CTGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCGAGCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAACTA CAAGACCACCG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

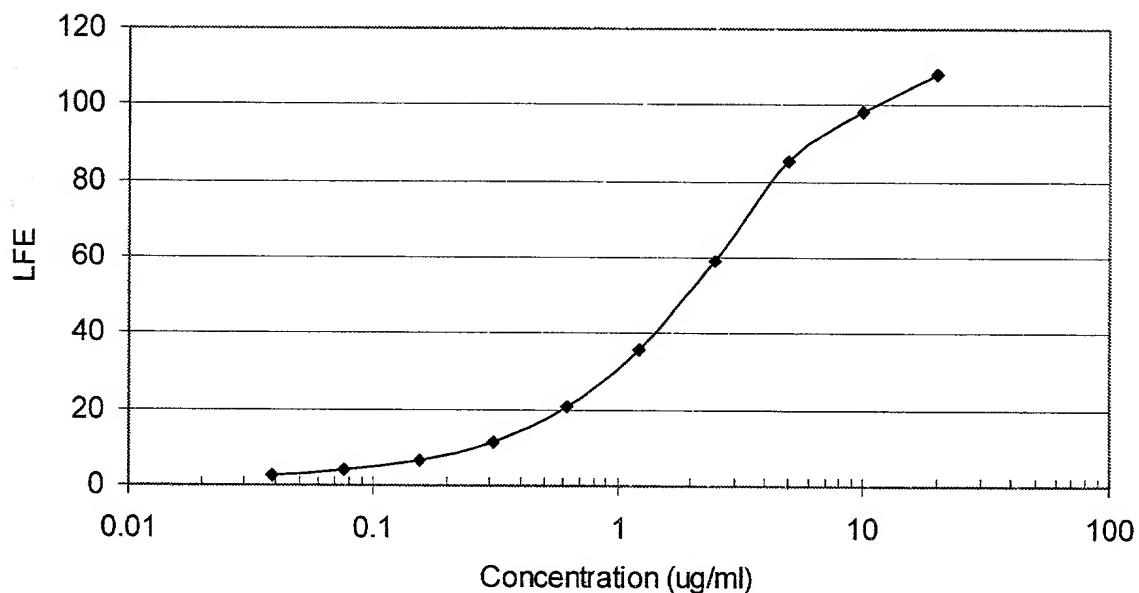
1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K * S R
CCGGGTAAAT **GATCTAGA**

Figure 2.

2H7scFvIg Standard Curve



Clone	LFE @ 1:50	Estimated Concentration (μg/ml)
D2	26.1	56
IIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

Figure 3.

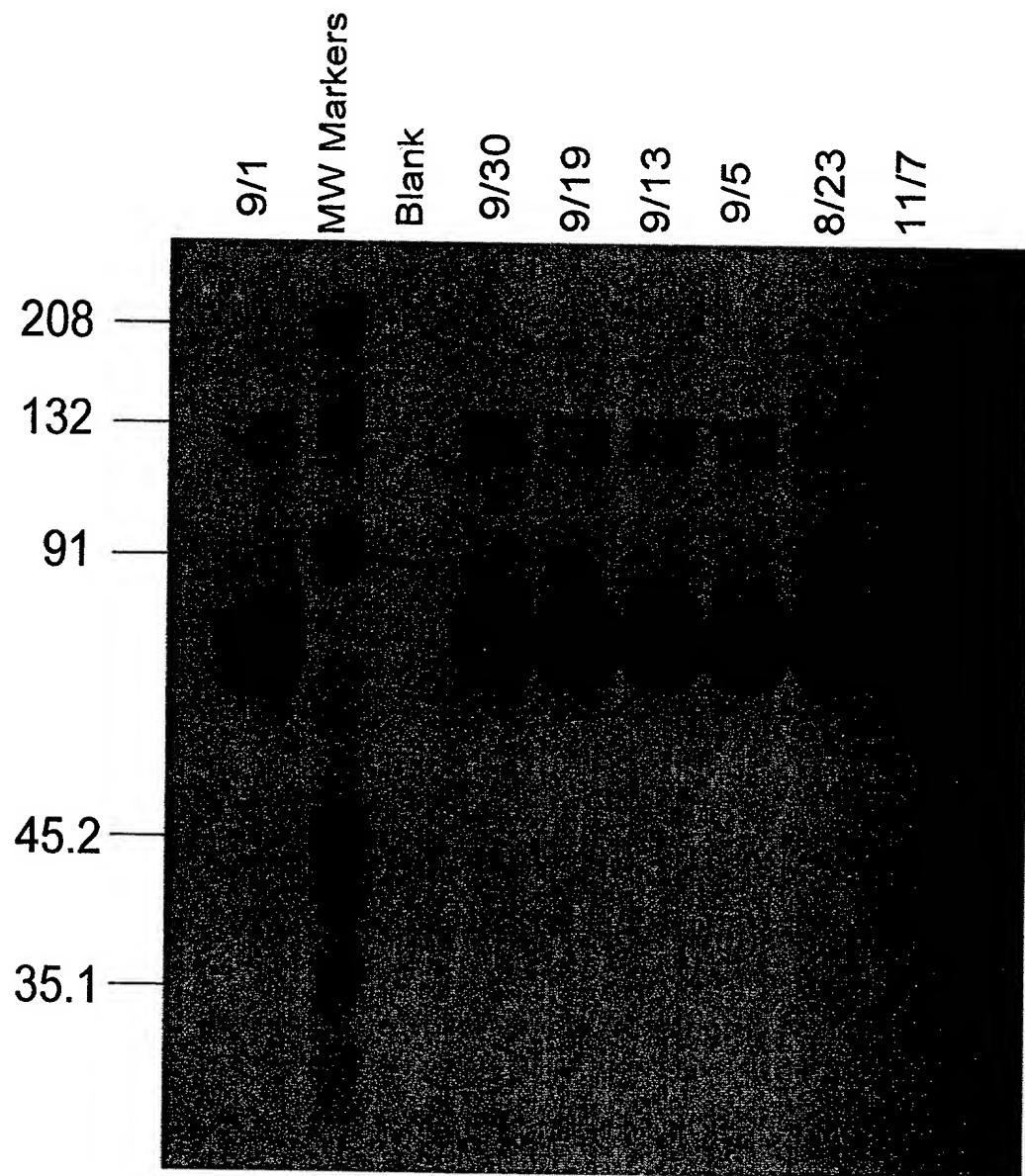


Figure 4A.

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Figure 4B.

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

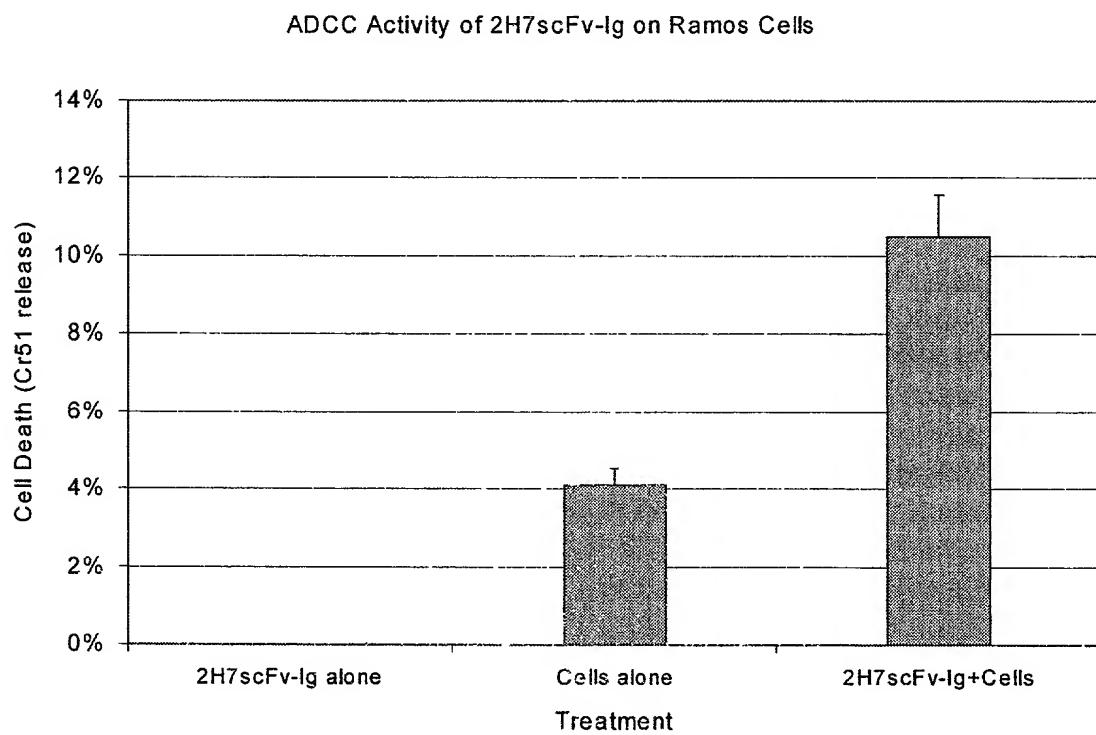


Figure 5.

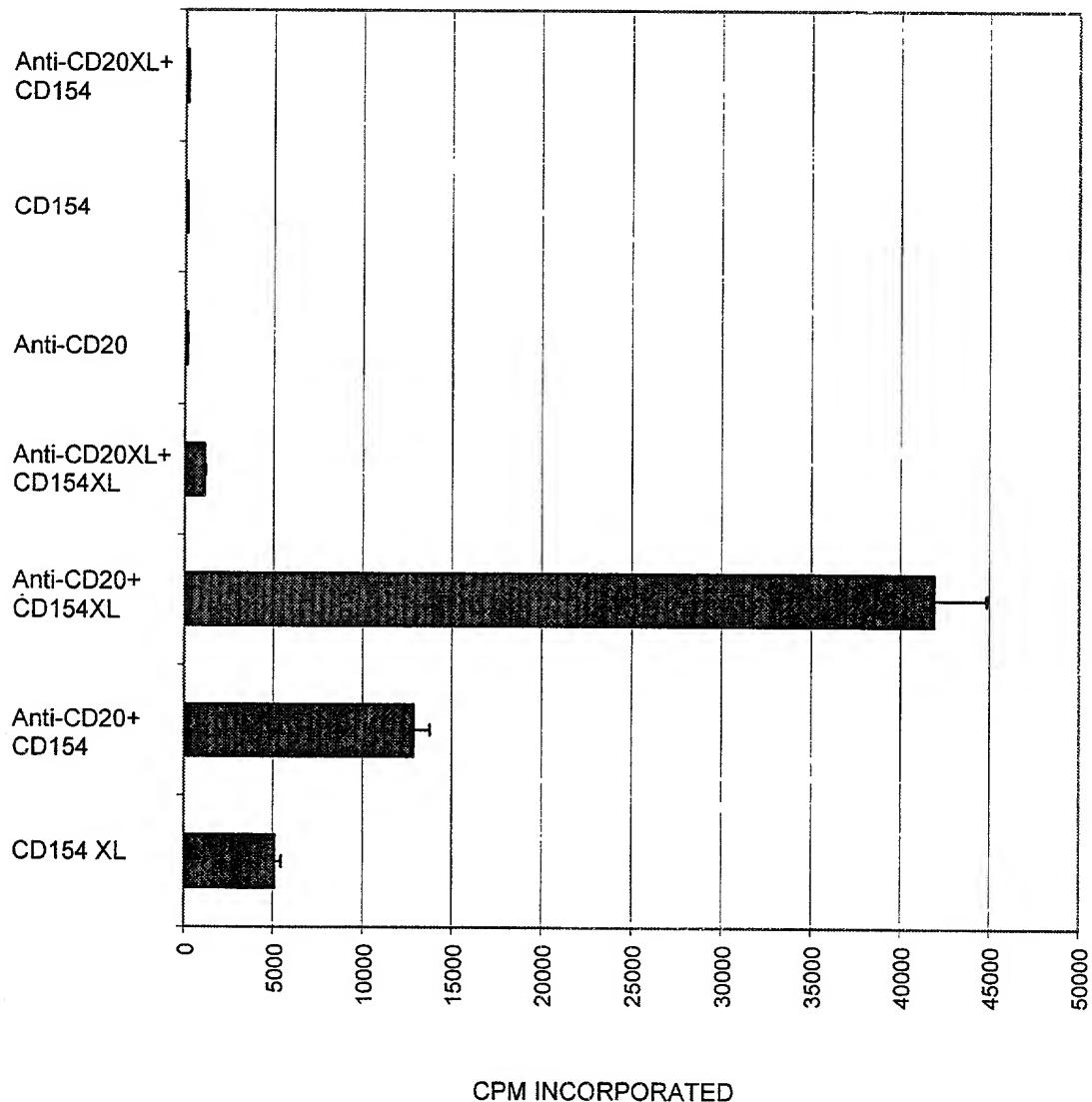
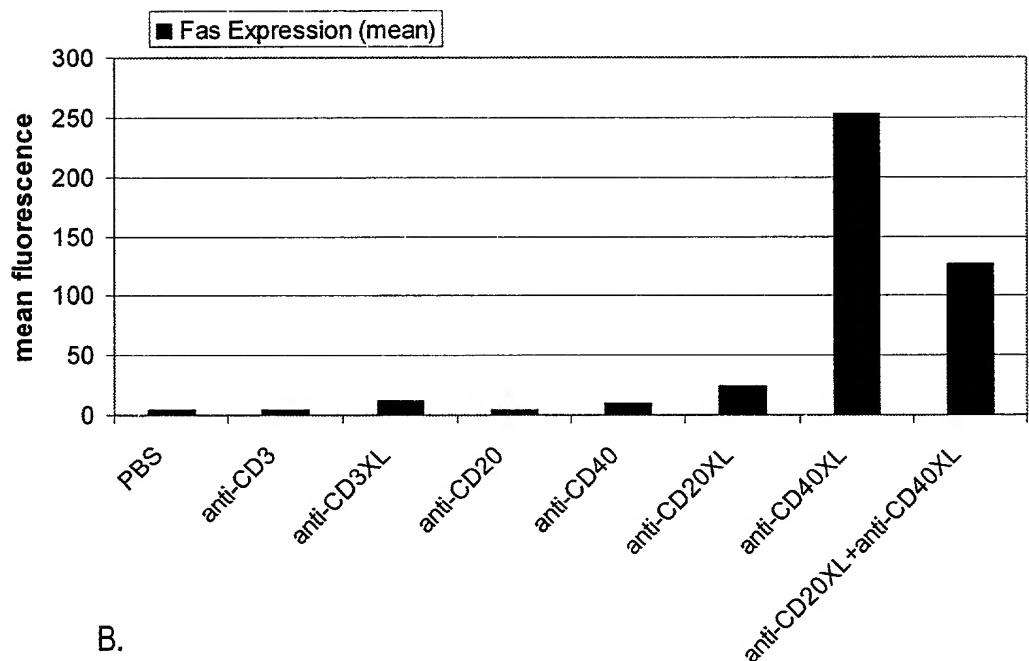


Figure 6A and B.

A.



B.

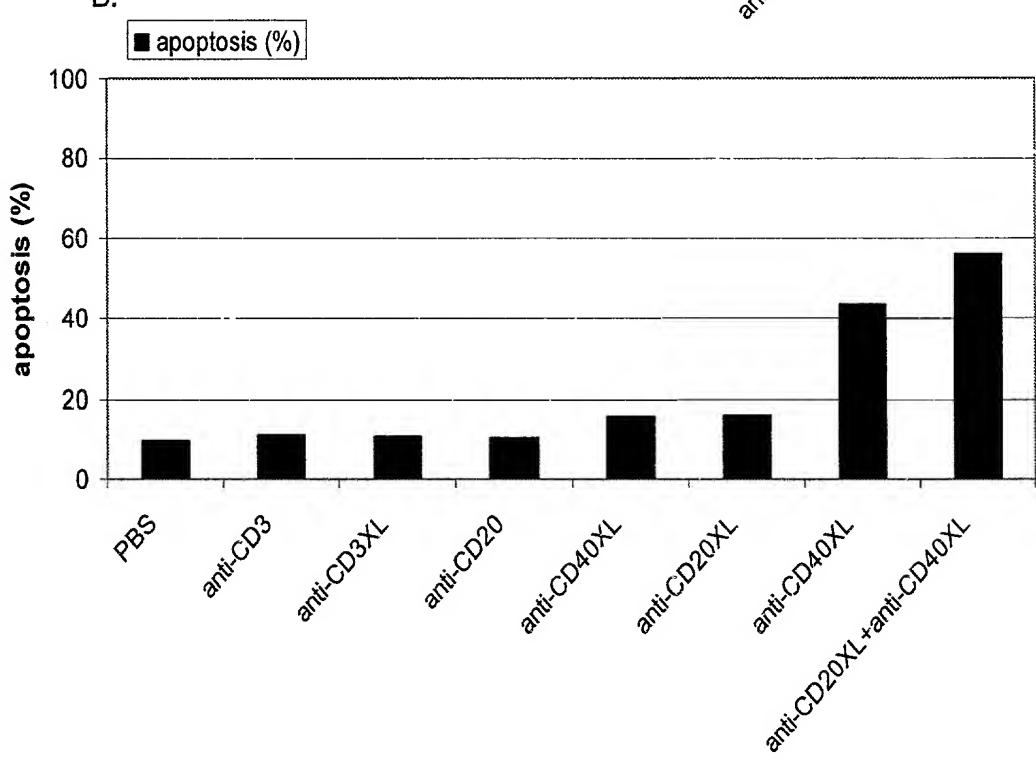


Figure 7A.

2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide →
~~~~~ ~~~~~~  
1    **AAGCTT**GCCG CC    ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA  
  
2H7 V<sub>L</sub> →  
V I I A R G Q I V L S Q S P A I L S A S  
61    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT  
  
P G E K V T M T C R A S S S V S Y M H W  
121   CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG  
  
BamHI  
~~~~~  
181 Y Q Q K P G S S P K P W I Y A P S N L A
TACCAAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTG ATGCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA

(Gly₄Ser)₃ Linker →
P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H →
G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTCT GTGCAAGAGT GGTGTACTAT ACTAACTCTT ACTGGTACTT CGATGCTGG

Figure 7B

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E
GGCACAGGGA CCACGGTCAC CGTCTCT**TGAT** CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E
AGGAATCTC ATGAAGATTT TGTATTCTATG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTGAAAT GCAAAAAGGT

BclI

~~~~~

1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGTT AACCTGGAA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCAGGC ACAATCCAT TCACTTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

NcoI

~~~~~

1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACGGCTT CACGTCTTT

XbaI

~~~~~

XbaI

~~~~~

1441 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAAT**CTAGA**

## Figure 7 C

### 2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

HindIII      NcoI  
~~~~~ ~~~~~~2H7 V<sub>L</sub> Leader Peptide→  
M D F Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V_L →
V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI
~~~~~  
Y Q Q K P G S S P K P W I Y A P S N L A  
181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I  
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

S R V E A E D A A T Y Y C Q Q W S F N P  
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA

(Gly<sub>4</sub>Ser)<sub>3</sub> Linker →  
P T F G A G T K L E L K G G G G S G G G  
361 CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT

2H7 V<sub>H</sub> →  
G S G G G S S Q A Y L Q Q S G A E L V  
421 GGATCTGGAG CAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y  
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y  
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT

P G N G D T S Y N Q K F K G K A T L T V  
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A  
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W  
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACCTT ACTGGTACTT CGATGTCTGG

## Figure 7D.

human CD154/amino acid 108 →

BclI/Bam hybrid site BclI  
781 G T G T T V T V S D P E N S F E M Q K G  
GGCACAGGGA CCACGGTCAC CGTCTCT**TGAT** CCAGAAACA GCTTGAAAT GCAAAAGGT

BclI  
~~~~~  
841 D Q N P Q I A A H V I S E A S S K T T S
GATCAGAAC GCTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

V L Q W A E K G Y Y T M S N N L V T L E
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII
~~~~~  
1021 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

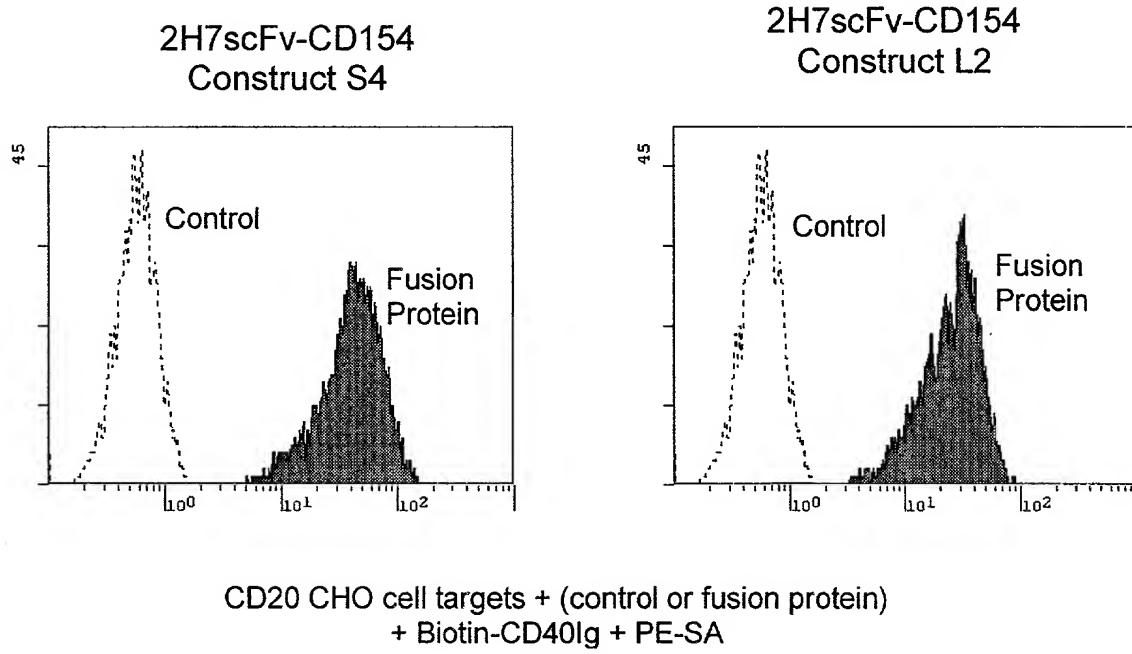
S P G R F E R I L L R A A N T H S S A K  
1081 TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

P C G Q Q S I H L G G V F E L Q P G A S  
1141 CCTTGCAGGC AACAAATCCAT TCACTTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

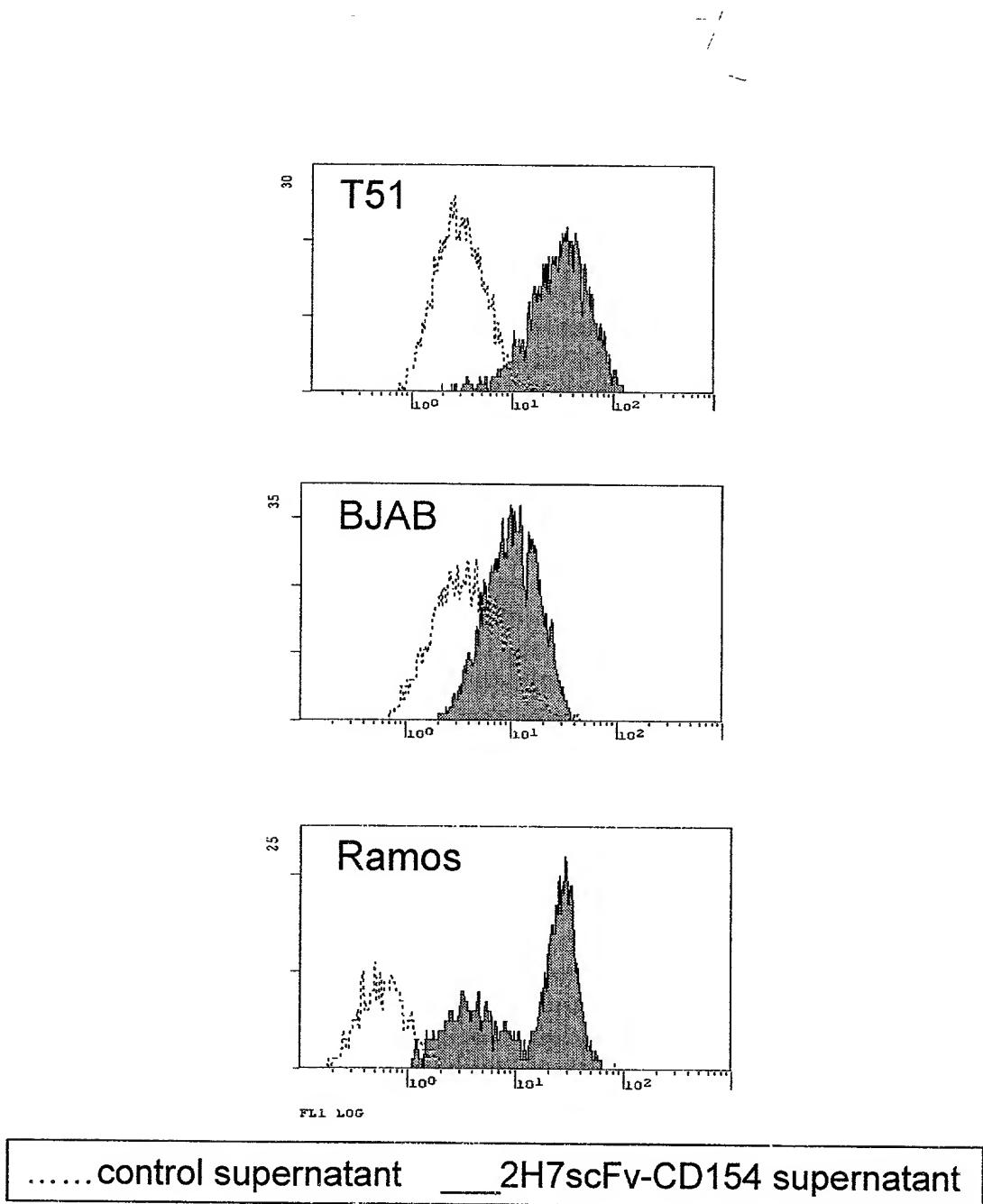
NcoI  
~~~~~  
1201 V F V N V T D P S Q V S H G T G F T S F
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCCTT

XhoI XbaI
~~~~~ ~~~~  
1261 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA

**Figure 8.**



**Figure 9.**



**Figure 10.**

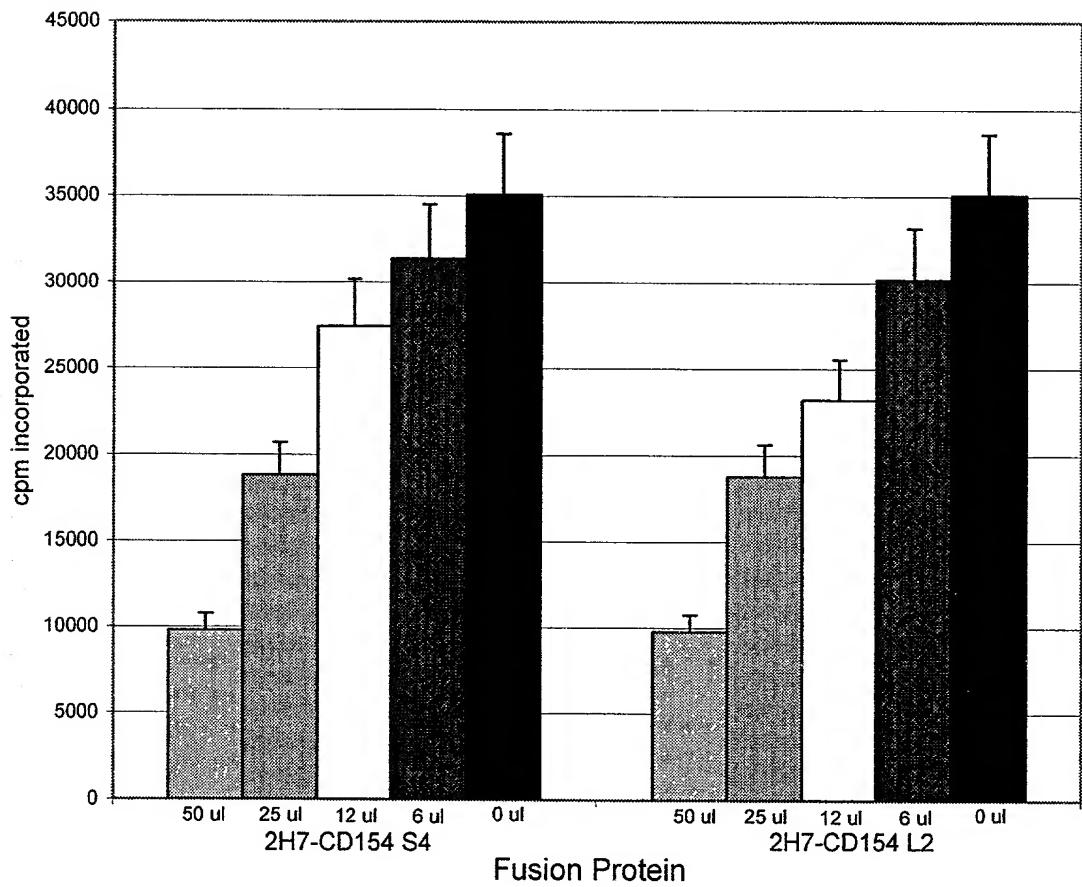
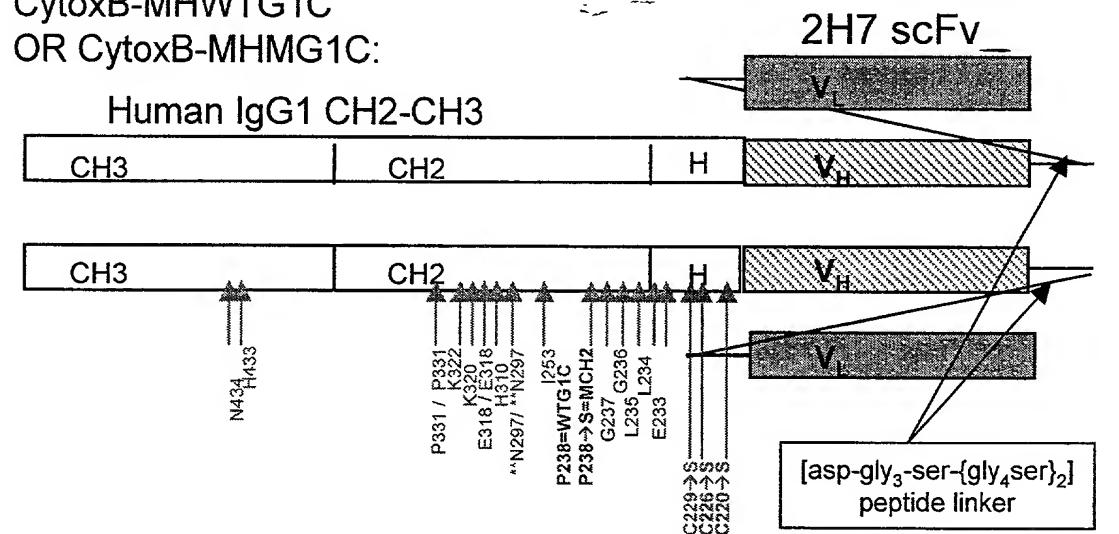


FIGURE 11

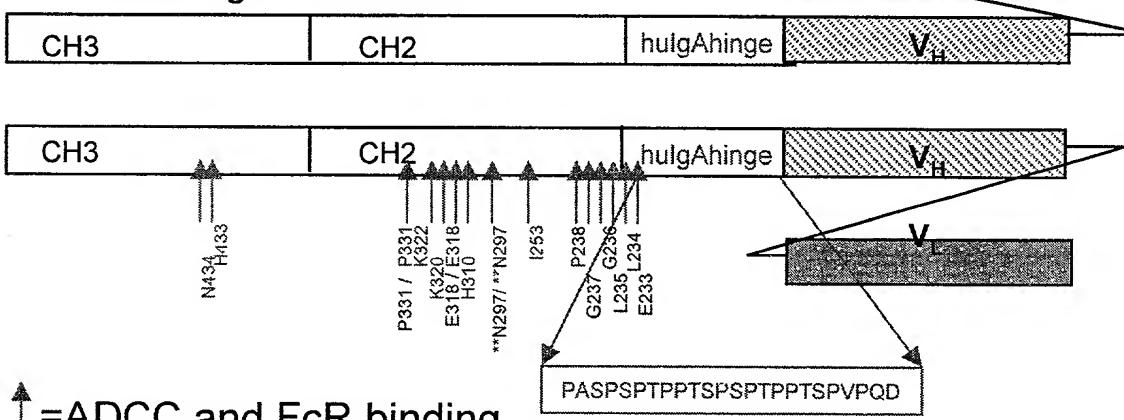
CytoxB-MHWTG1C  
OR CytoxB-MHMG1C:

Human IgG1 CH2-CH3



CytoxB-IgAHWTHG1C:

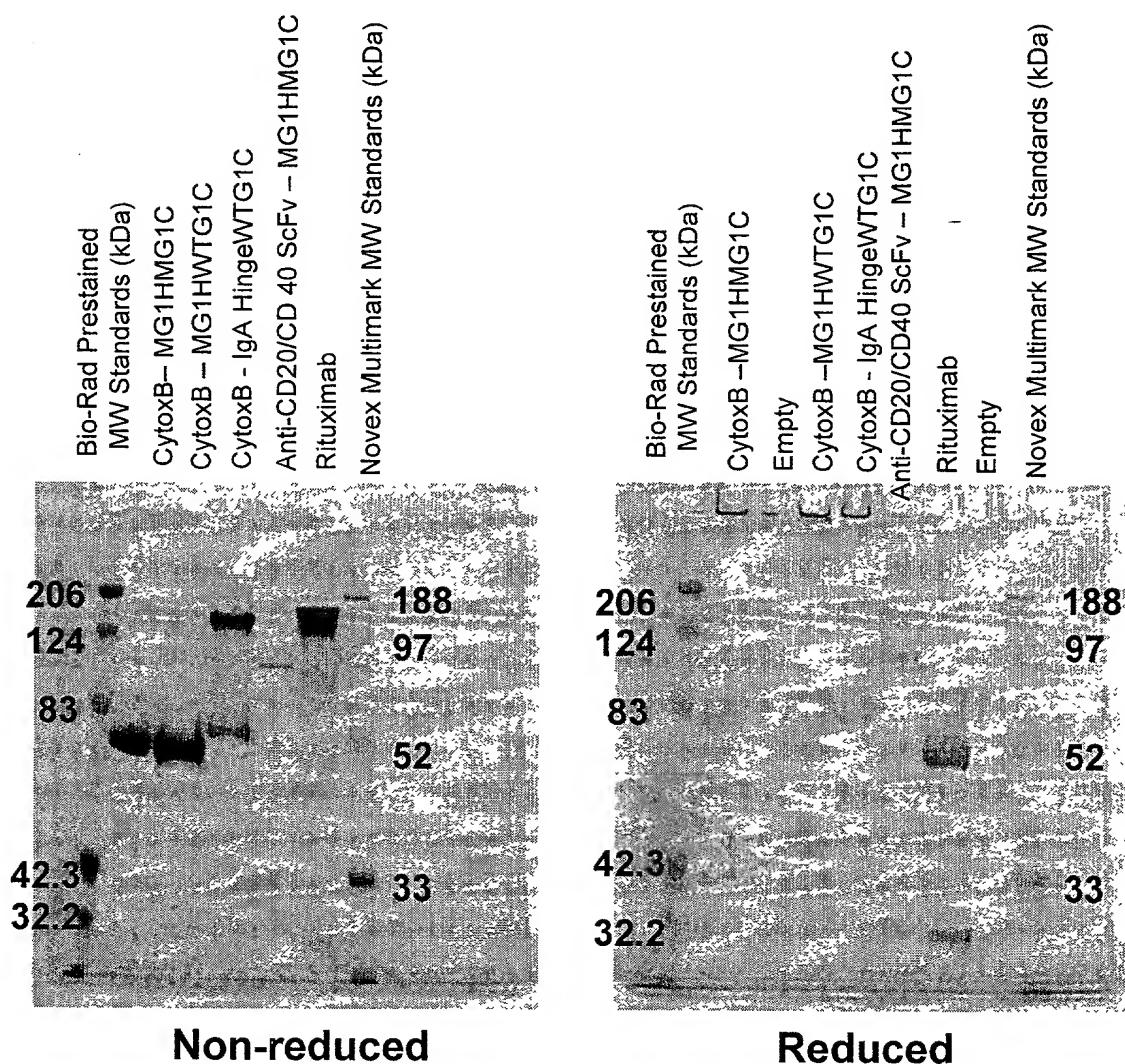
Human IgG1 CH2-CH3



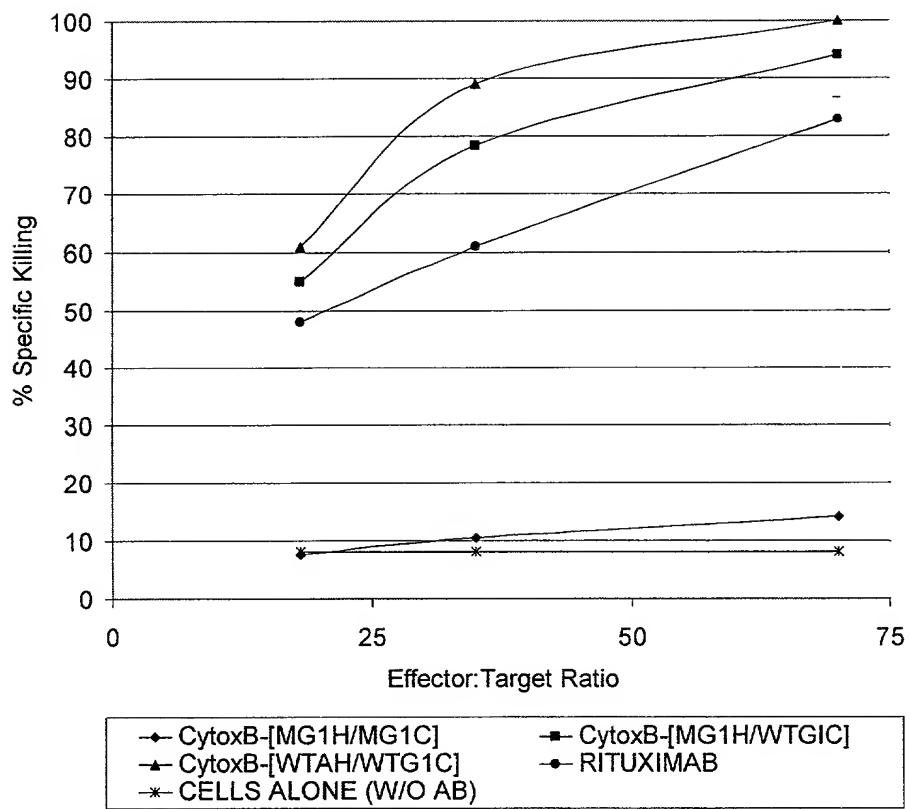
↑ =ADCC and FcR binding

↑ =Complement Fixation

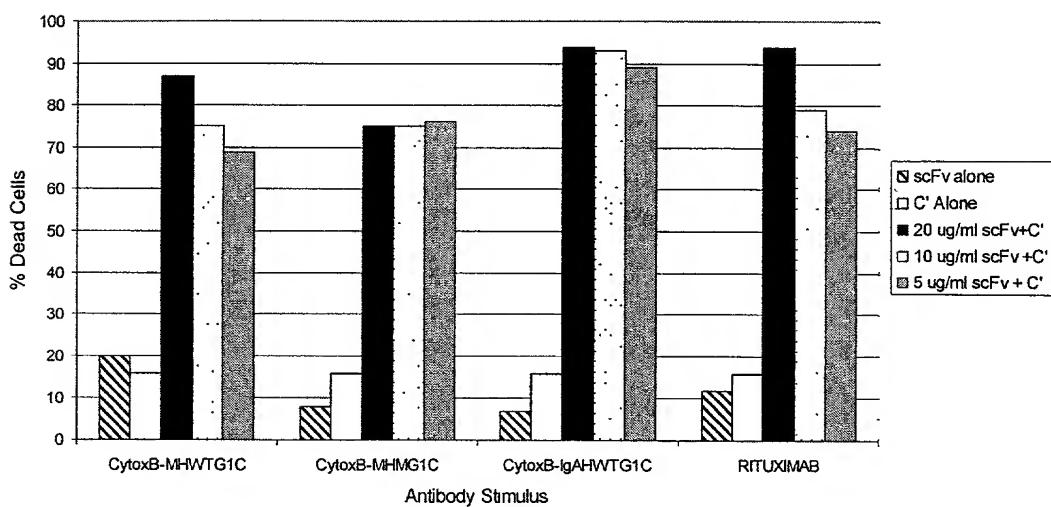
**FIGURE 12**



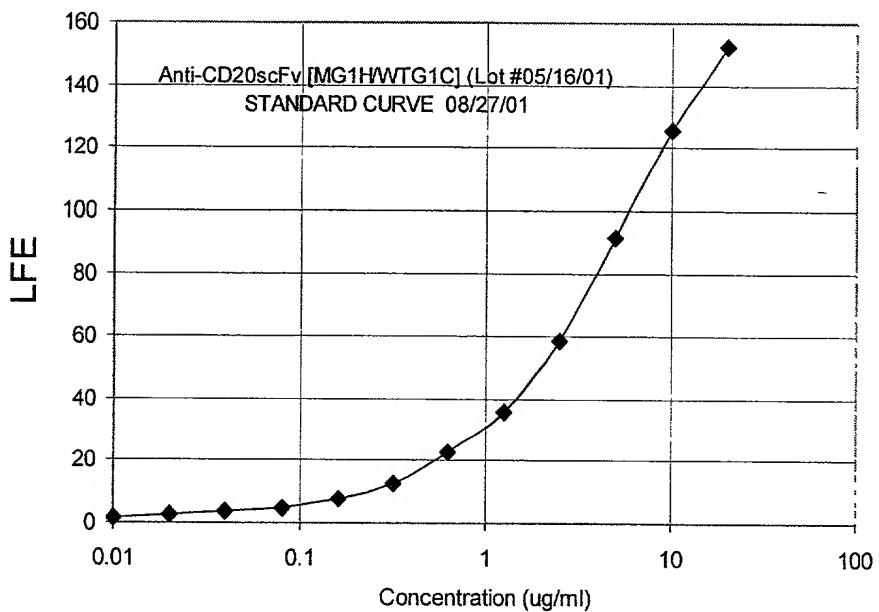
**FIGURE 13**



**FIGURE 14**



**TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS**  
Inventors: Jeffrey A. Ledbetter et al. Docket No. 390069.401  
EXPRESS MAIL NO. EL 755733415US  
FIGURE 15



| Day                     | Monkey J99231 |                                | Monkey K99334   |                                |
|-------------------------|---------------|--------------------------------|-----------------|--------------------------------|
|                         | LFE(1:40)     | Concentration<br>( $\mu$ g/mL) | LFE(1:40)       | Concentration<br>( $\mu$ g/mL) |
| Injection $\rightarrow$ | 7             | 2.41                           | <0.6 $\mu$ g/mL | <0.4 $\mu$ g/mL                |
|                         | 0             | 2.22                           | <0.6 $\mu$ g/mL | <0.4 $\mu$ g/mL                |
| Injection $\rightarrow$ | 1             | 73.8                           | 220 $\mu$ g/mL  | 100 $\mu$ g/mL                 |
|                         | 3             | 20.0                           | 28 $\mu$ g/mL   | 80 $\mu$ g/mL                  |
|                         | 7             | 15.6                           | 24 $\mu$ g/mL   | 24 $\mu$ g/mL                  |
|                         | 8             | 39.1                           | 80 $\mu$ g/mL   | 92 $\mu$ g/mL                  |
|                         | 10            | 11.5                           | 18 $\mu$ g/mL   | 1.2 $\mu$ g/mL                 |
|                         | 14            | 2.05                           | 0.6mg/mL        | 0.6 $\mu$ g/mL                 |

**Figure 16**

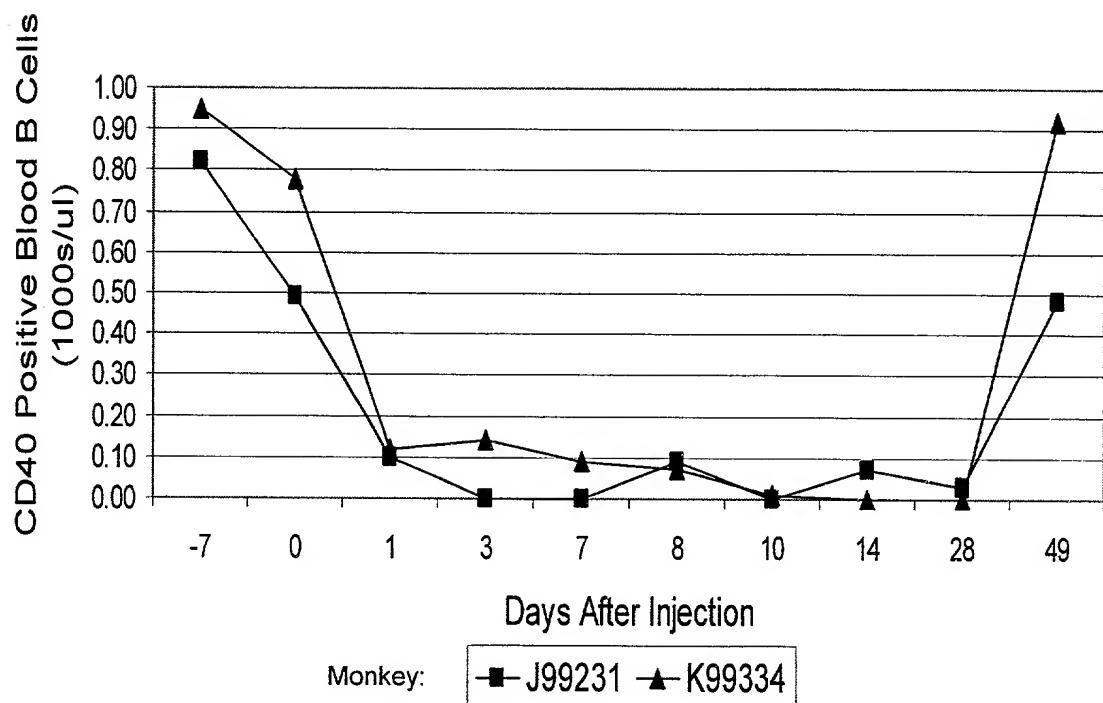
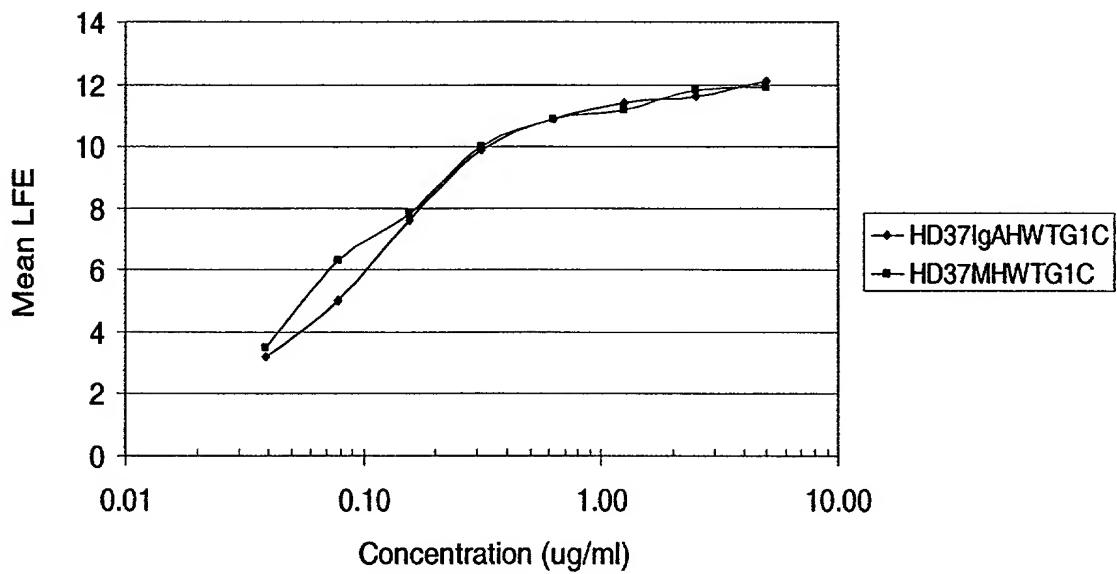


FIGURE 17

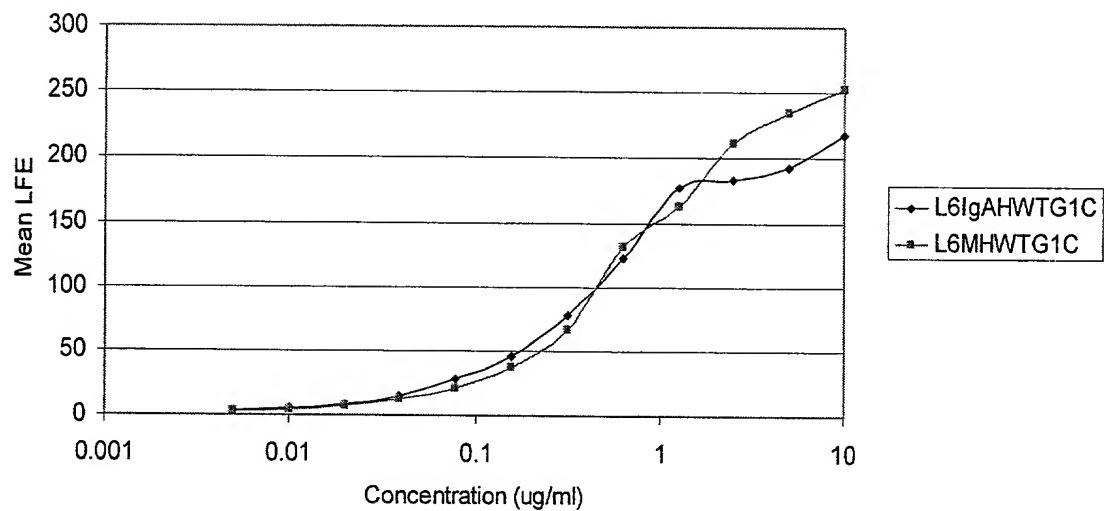
Standard Curve of HD37 scFvIg Derivative  
Binding to B Cells



| Clone/Isolate  | Mean LFE at 1:100 | Estimated Concentration |
|----------------|-------------------|-------------------------|
| Bulk IgAHWTG1C | 11.2              | > 60 ug/ml              |
| 1B2            | 10.4              | >50 ug/ml               |
| 6C5            | 10.5              | >50 ug/ml               |
| 4B1            | 8.6               | >40 ug/ml               |
| Bulk MHWTG1C   | 10.9              | > 50 ug/ml              |
| 2G8            | 10.6              | > 50 ug/ml              |
| 3F3            | 8.3               | >40 ug/ml               |
| 3D9            | 11.1              | > 60 ug/ml              |

FIGURE 18

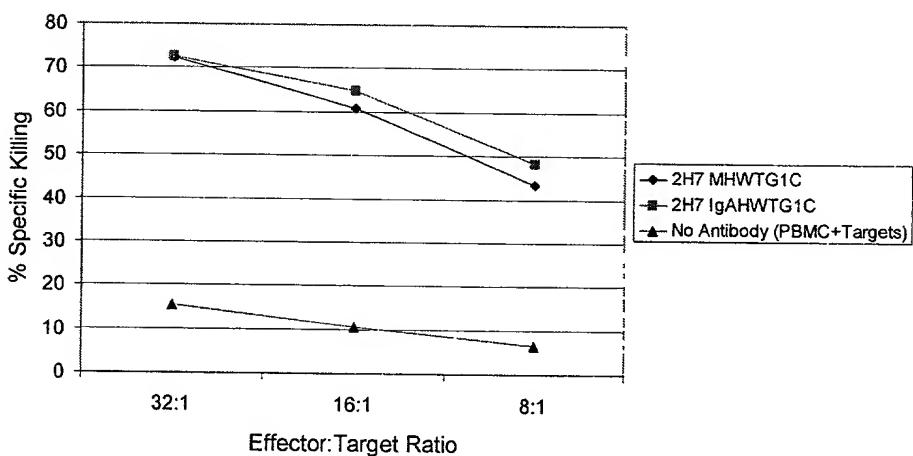
L6 scFvIg Standard Curves



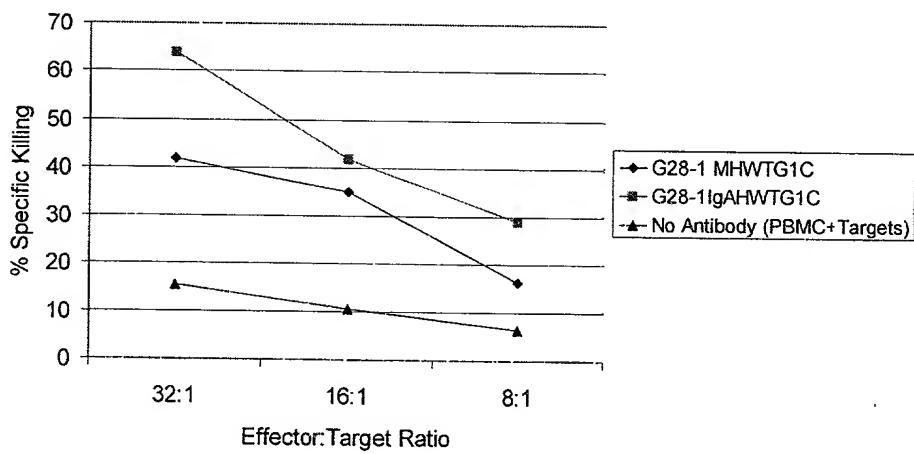
| Construct                           | Mean LFE 1:20 | Estimated Concentration |
|-------------------------------------|---------------|-------------------------|
| L6IgAHWTG1C<br>unamplified CHO sup  | 51.1          | 6.25 ug/ml              |
| L6IgGMHWTG1C<br>unamplified CHO sup | 23.0          | 3.2 ug/ml               |

FIGURE 19

**A. 2H7 (anti-CD20) scFv Derivatives**



**B. G28-1 (anti-CD37) scFv Derivatives**



**C. HD37 (anti-CD19) scFv Derivatives**

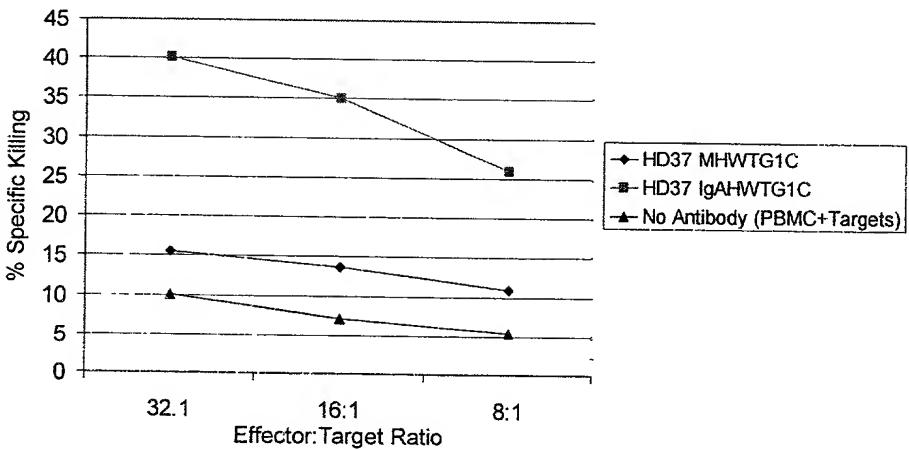


FIGURE 20

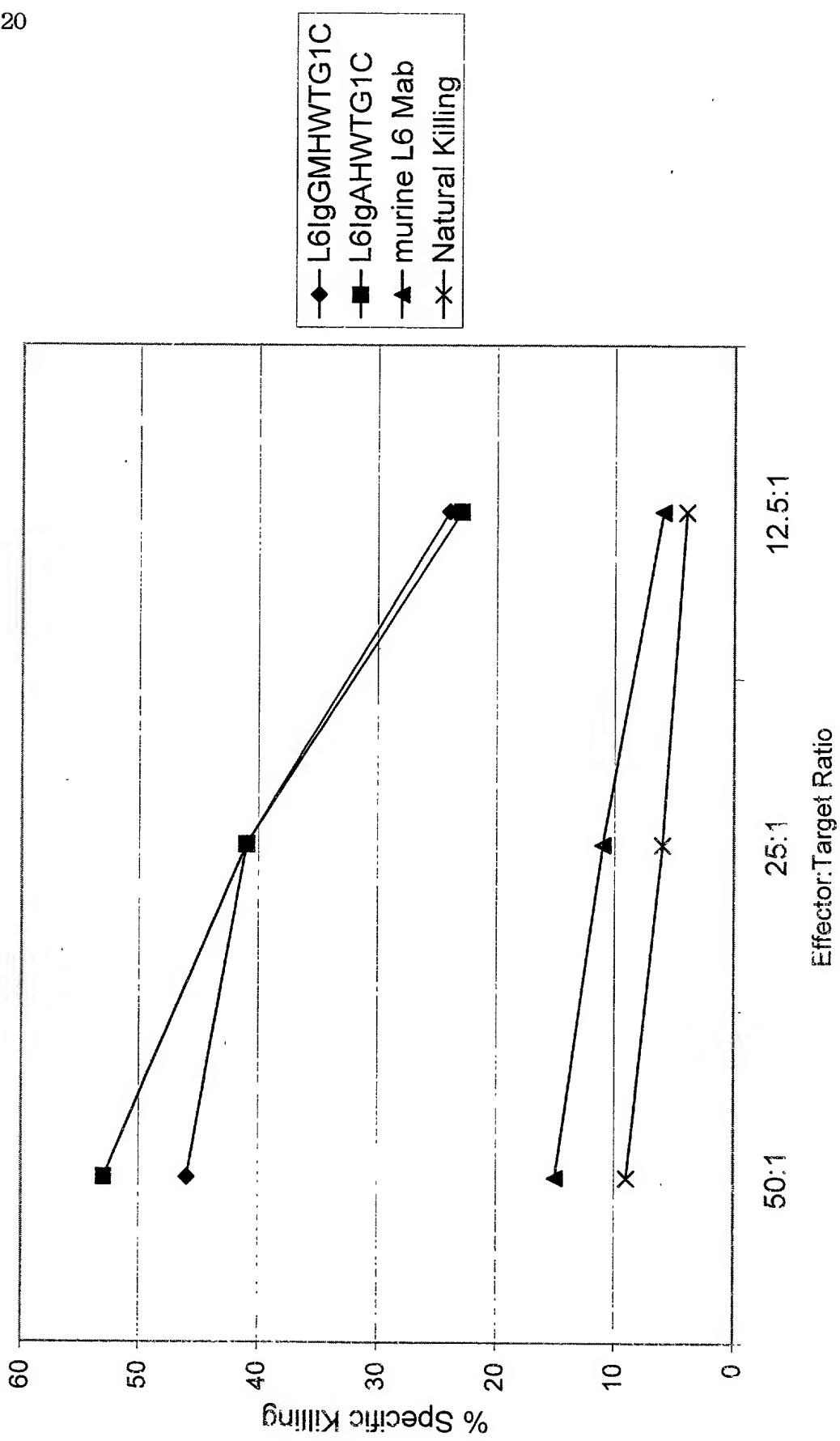


Figure 21

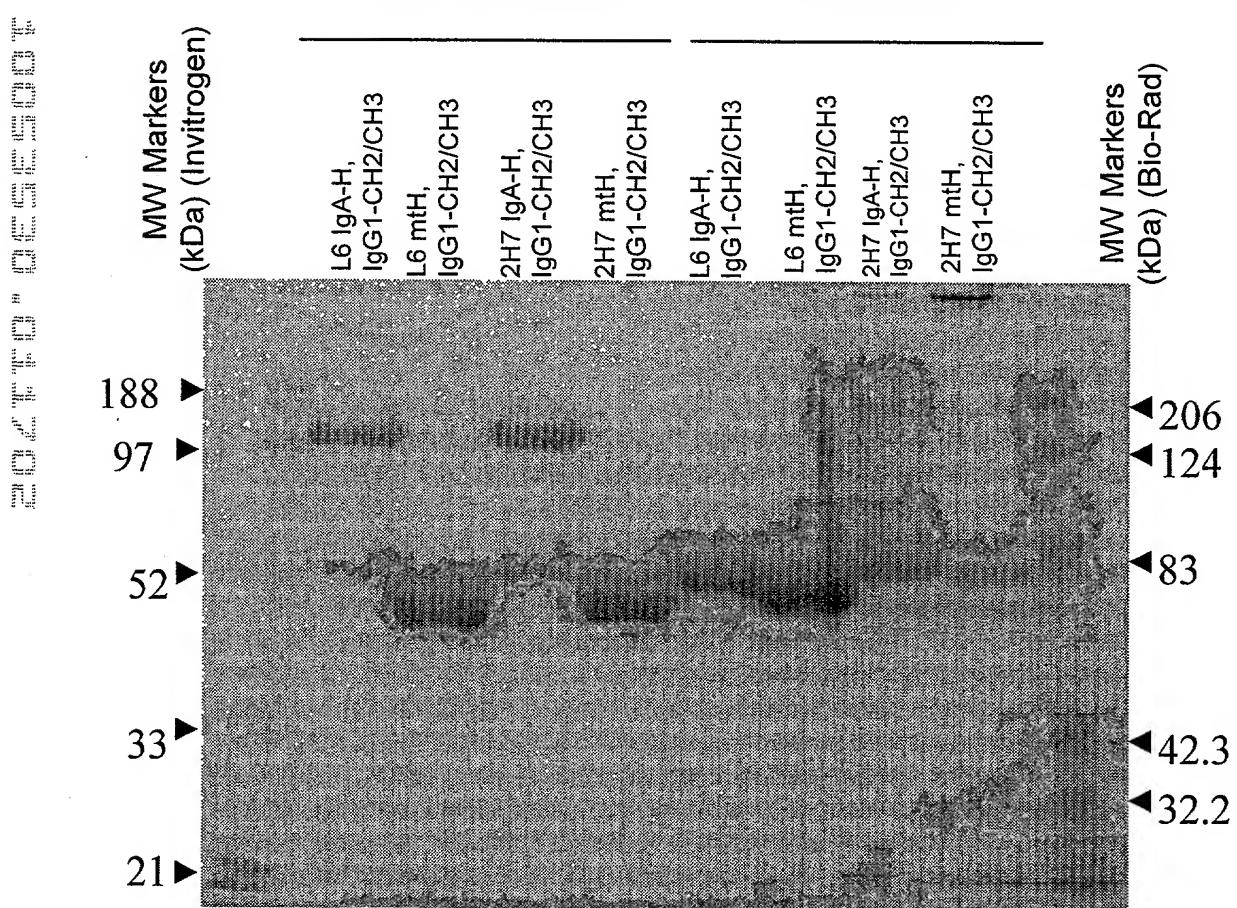


Figure 22

